Phylogenetic analysis of click beetles (Coleoptera: Elateridae) based upon 28S rDNA: phylogeny and classification

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Abstract: Elateridae (click beetles) is the largest family within the Elateroidea and is abundant worldwide. However, their phylogenetic relationships remain uncertain; older studies based on the external morphology of adults and larvae led to divergent classification systems. Many tribes, genera, and even subfamilies remain controversial. Partial sequences of 28S ribosomal DNA (rDNA) were used to investigate the phylogeny of click beetles. Sequences of 28S rDNA from 80 different click beetle species collected from different locations across China were determined along with those of another 86 Elateridae species and 14 outgroup species. The aligned data were analyzed by neighbor joining, maximum parsimony, maximum likelihood, and Bayesian analyses. All methods produced a nearly consistent basal topology that was partially congruent with those of previous studies based on adult and larval morphology as well as molecular data. Overall, the monophyly of Elateridae was supported. The family was divided into five clades, Denticollinae, Negastriinae, Cardiophorinae, Pyrophorinae, Oestodinae, and Elaterinae. *Senodonia* is traditionally considered a member of Elateridae, but this study indicated that it was more closely related to Lycidae. The position of the outgroup family Lampyridae also differed from its placement in previous research. In addition to confirming the Elateridae as monophyletic, we concluded that Oxynopterinae, Pityobiinae, and Hypnoidinae should be merged into Denticollinae; Conoderinae and Agrypninae should be regarded as subgroups of Pyrophorinae; and Melanotinae should be classified as a tribe of Elaterinae. Additional species should be sampled in future studies to confirm this reclassification.

Key words: Elateroidea; classification systems; taxonomy

基于 28S rDNA 片段的叩甲科昆虫系统发育分析：系统发育与分类（鞘翅目：叩甲科）

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摘要：叩甲科昆虫是叩甲总科中最大的一科，广泛分布于全世界。但是直到现在叩甲科的分类系统还

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存在着不确定的地方，因为早期的分类系统是基于成虫和幼虫的外部形态特征建立的，很多的族、属、甚至亚科的划分都有争议。本研究利用核糖体 28S rDNA 片段，对国内采集的叩甲科昆虫共 80 种进行系统发育分析。分析中还采用了 GenBank 已有的 86 种叩甲科昆虫序列作为内群，14 种外群，利用邻接法、最大似然法、最大简约法和贝叶斯法构建系统发育树。不同方法构建的系统发育树结构相同，并部分支持了基于外部形态特征建立的分类系统。结果显示，叩甲科共分为 5 个分支，分别为齿胸叩甲亚科分支、小叩甲亚科分支、心盾叩甲亚科分支、萤叩甲亚科分支以及叩甲亚科分支。其中，方胸叩甲属属于叩甲科类群，在系统树中却更加接近于红萤科，并且外群中萤科的位置也与以前的观点不同。研究的结果支持叩甲科昆虫具有良好的单系性。并且根据结果提出以下假设：尖鞘叩甲亚科、异角叩甲亚科和胖叩甲亚科可以纳入齿胸叩甲亚科内部；单叶叩甲亚科和槽缝叩甲亚科并入萤叩甲亚科；梳爪叩甲亚科并入叩甲亚科。这个结论还需要引入更多的种类及分子标记来加以证实。

关键词：叩甲总科；分类系统；分类

Introduction

The Elateridae, commonly termed click beetles, is comprised of a large number of insects belonging to the Coleoptera. Approximately 9,300 species of this insect family have been described and specimens can be found worldwide. They have distinctive characteristics. The adults are characterized by an elongated and narrow body, and the joint formed by the prothorax and meditruncus has become a unique defense mechanism (Lawrence & Newton 1982). Most adults of the elateriform lineage are ground surface-active, while the larvae live underground. Some beetle families possess a similar life-cycle. The Elateriformia lineage has been the subject of many research reports (Bocakova *et al*. 2007; Kundrata & Bocak 2011) but only a small fraction of Elateridae have been cytogenetically analyzed (Costa 2000).

Species of Elateridae present a wide range of differentiation and Elateridae taxonomists from various countries have developed different categorization systems for this family. In 1759, Linnaeus first established the Elater category. Subsequently, both the Elateridae and Buprestidae were classified into the Sternoxia taxon of Coleoptera (Latreille 1810, 1834). Later, Eschscholtz divided the click beetle family into 32 subfamilies, including Eucnemidae (Eschscholtz 1830), while LaPorte de Castelnau reported 58 subfamilies within this family (LaPorte de Castelnau 1836). Germar and Lacordaire amended and complemented the Eschscholtz system (Germar 1839; Lacordaire 1857). Candèze later divided the Elateridae into eight tribes and removed Eucnemidae from the family (Candèze 1857, 1859, 1860, 1863). This system was then upgraded to include 28 tribes (Schwarz 1906) and subsequently, to elevate all tribes to the subfamily level (Schenkling 1925, 1927).

The basis for classification also changed over time. For instance, some taxonomists used the external morphological features of larvae in click beetle phylogenetic studies (Hyslop 1917; Ohira 1962), whereas others revised the Elateridae taxonomy based on the abdomen shape and male genitalia structure of adult specimens (Jeannel & Paulian 1944). Later, taxonomists combined both larval and adult morphological features to perform phylogenetic studies and establish different categorization systems (Crowson 1960; Stibick 1979). Although the scholars engaged in the morphological classification of click beetles all conducted comprehensive studies of the external features of larvae and adults, they failed to address problems with the morphological identification of click beetles (Lacordaire 1857; Kishii 1987).

Only the species of the Agrypninae, Cardiophorinae, Denticollinae, and Elaterinae subfamilies have known chromosomal characteristics. Furthermore, two phylogenetic trees based on morphology can differ from each other (Stibick 1979; Ohira 1999). Altogether, these primarily morphological studies revealed a lack of consensus on the taxonomic classification of Elateridae family. Which external morphological characteristics are used to reconstruct evolutionary histories depended largely on the researcher's experience and subjective judgment, leading to conflicting viewpoints when classification was based on external morphology alone.

Recently, gene sequences have been applied as molecular markers to taxonomically identify click beetles. Thus far, phylogenetic analyses of Elateridae and Elateriformia based on sequences of 28S and 18S ribosomal DNA (rDNA) and the mitochondrial *rrnl* and *cox1* genes have been reported. Kundrata and Bocak analyzed the phylogeny of Elateroidea using these molecular markers and concluded that Drilidae and Cebrioninae belonged to Elateridae (2011); this proposal was supported by Bocakova *et al*. (2007). 28S rDNA has both a conserved and a variable region that makes it suitable for the phylogenetic analysis of eukaryotes, including insects (Gillespie *et al*. 2004). In previous studies based on both morphology and molecular systematics, the monophyly of Elateroidea was well supported. However, in early morphological studies of Elateridae, the family appeared to be paraphyletic (Calder *et al*. 1993; Muona 1995). In contrast, molecular phylogenetic analyses strongly support the monophyly of Elateridae (Bocakova *et al*. 2007; Kundrata & Bocak 2011; Sagegami-Oba *et al*. 2007; Hunt *et al*. 2007; Bocak *et al*. 2014).

The current classification of the click beetles of China is mostly based upon the system established by Kishii using morphological features (1987) which contradicts other classification systems that appeared later. Thus, the phylogeny of Elateridae must be revisited to get an accurate taxonomy for all of its members. We conducted a phylogenetic analysis of some of the Chinese Elateridae groups using modern molecular methods and we discuss our results in light of the classification and external morphological features of these beetles.

Material and methods

Specimen collection and storage

Specimens of Elateridae were collected from the Chinese mainland. All specimens used in this study are deposited in the Insect Lab of Shenzhen Polytechnic, Guangdong Province, China. Our samples represent 80 species from 12 subfamilies of Elateridae. We also used 28S rDNA sequence data of 86 other Elateridae species and 14 outgroup species downloaded from GenBank by Sagegami-Oba *et al*. (2007) and Jiang *et al*. (2009). Of these taxa, *Pyrophorus clarus*, *Pyrophorus noctilucus*, *Pyrophorus pisticus*, and *Phanophorus perspicax* were from Central and South America because they are restricted to the Neotropical Region. The sampled categories and collection sites are shown in Figure 1 and Table 1. Collected samples were immediately immersed in absolute ethyl alcohol and stored at −20°C until use.

DNA extraction and storage

Total DNA was extracted as previously described with some modifications (Philips & Simon 1995). Total DNA was extracted from one of the beetle's rear legs and stored at −20 °C until use.

Table 1. Checklist of elaterid species with collection locations and references shown in this study (Taxonomy follows Kishii 1987 based on the primarily external morphological characteristics)

Taxa	GenBank No.	Collection location or reference
Oxynopterinae		
Ceropectus messi (Candèze, 1874)	JF713740	China: Zhejiang, Zhenbu
Campsosternus auratus (Drury, 1773)	JF713737	China: Guangdong, Shenzhen, Mt. Wutong
Campsosternus fruhstorferi (Schwarz, 1902)	JF713738	China: Guangxi, Jinxiu, Tongmuzhen
Campsosternus matsumurae Miwa, 1929	AB231227	Sagegami-Oba *et al*., 2007
Agrypninae		
Agrypnus judex (Candèze, 1874)	JF713759	China: Hubei, Guangshui, Daguisi
Agrypnus acuminipennis (Fairmaire,1878)	JF713728	China: Sichuan, Ya'an, Baoxin, Longdong
Agrypnus montanus (Miwa, 1929)*	EU677185	Jiang *et al*., 2009
Agrypnus sinensis (Candèze, 1857)	JF713733	China:Yunnan, Dali, Mt.Cang
Agrypnus musculus (Candèze, 1857)	JF713791	China: Sichuan, E'bian, Heizhugou
Agrypnus gypsatus (Candèze, 1891)	JF713731	China: Sichuan,Ya'an, Baoxing, Fengtongzhai
Agrypnus kawamurae (Miwa, 1929)	JF713732	China: Hannan, Mt. Wuzhi
Agrypnus argillaceus (Solsky, 1870)	JF713729	China: Sichuan, E'bian, Heizhugou
Agrypnus bipapulatus (Candèze, 1865)	JF713730	China: Guangxi, Lingui, Huangsha
Agrypnus baibaranus (von Hayek, 1973)*	EU677187	Jiang *et al*., 2009
Rismethus ryukyuensis (Ôhira, 1999)*	AB231281	Sagegami-Oba *et al*., 2007
Agrypnus binodulus binodulus (Motschulsky, 1861)	AB231218	Sagegami-Oba *et al*., 2007
Agrypnus fuliginosus (Candèze, 1865)	AB231219	Sagegami-Oba *et al*., 2007
Agrypnus scrofa scrofa (Candèze, 1873)	AB231220	Sagegami-Oba *et al*., 2007
Pyrophorinae		
Adelocera tumens (Candèze, 1873)	JF713723	China: Guangxi, Jinxiu, Mt.Huawang
Alaus sculptus (Westwood,1848)	JF713734	China: Guangdong, Mt. Nanling
Lacon rotundicollis (Kishii et Jiang, 1994)	JF713758	China: Guangxi, Guilin, Lengshuitang
Lacon sp.	JF713757	China: Yunnan, Dali, Mt.Cang
Tetralobus perroti (Fleutiaux, 1940)	JF713787	China: Guangdong, Zhaoqing, Heishiding
Tetralobus perroti (Fleutiaux, 1940)	JF713773	China: Guangdong, Mt. Nanling
Cryptalaus larvatus (Candèze, 1874)*	EU677188	Jiang *et al*., 2009
Cryptalaus berus (Candèze, 1865)	JF713743	China: Yunan, Xishuangbanna, Menglun
Cryptalaus sordidus (Westwood, 1848)	JF713744	China: Zhejiang, Zhenbu
Adelocera diffcilis (Lewis, 1894)	AB231216	Sagegami-Oba *et al*., 2007
Tetrigus lewisi Candèze, 1873	AB231286	Sagegami-Oba *et al*., 2007
Cryptalaus berus (*Candèze, 1865*)	AB231232	Sagegami-Oba *et al*., 2007
Cryptalaus larvatus pini (Lewis, 1894)	AB231233	Sagegami-Oba *et al*., 2007
Phanophorus perspicax (Guérin, 1830)	AB231268	Sagegami-Oba *et al*., 2007
Pyrophorus clarus (Germar, 1841)¤	AB231276	Sagegami-Oba *et al*., 2007
Pyrophorus noctilucus (Linnaeus, 1758)	AB231277	Sagegami-Oba *et al*., 2007
Pyrophorus pisticus (Costa, 1972)	AB231278	Sagegami-Oba *et al*., 2007
Conoderinae		
Conoderus tonkinensis (Fleutiaux, 1894)	JF713742	China: Guangxi, Jinxiu, Mt. Lao
Aeoloderma brachmana (Candèze, 1859)	JF713724	China: Hubei, Shennongjia
Heterodores albicans (Candèze, 1878)	JF713754	China: Sichuan, Ya'an, Baoxin, Longdong
Heterodores changi (Ôhira, 1967)	JF713755	China: Guangdong, Shenzhen, Mt. Wutong
Herteroderes macroderes (Candèze, 1859)	JF713756	China: Guangxi, Xing'an, Gaozhai
Prodrasterius brahminus (Candèze, 1859)	JN561610	China: Sichuan, Ya'an, Baoxing

Continued Table 1

Taxa	GenBank No.	Collection location or reference
Prodrasterius sp.	JN561611	China: Sichuan, Leshan, Mabian
Prodrasterius agnatus (Candèze, 1873)	AB231274	Sagegami-Oba *et al*., 2007
Conoderus sp.	AB231230	Sagegami-Oba *et al*., 2007
Pityobiinae		
Pectocera fortunei (Candèze, 1929)	JF713776	China: Guizhou, Guiyang
Pectocera fortunei fortunei (Candèze, 1873)*	AB231267	Sagegami-Oba *et al*., 2007
Hypnoidinae		
Homotechnes corymbitoides (Candèze, 1881)	JN561604	China: Yunnan, Nujiang, Luzhang
Ascoliocerus fluviatilis (Lewis, 1894)	AB231224	Sagegami-Oba *et al*., 2007
Ascoliocerus saxatilis saxatilis (Lewis, 1894)*	AB231225	Sagegami-Oba *et al*., 2007
Homotechnes brunneofuscus (Nakane, 1954)*	AB231248	Sagegami-Oba *et al*., 2007
Hypolithus littoralis convexus (Miwa, 1928)*	AB231249	Sagegami-Oba *et al*., 2007
Denticollinae		
Pristilophus sp.	JN561608	China: Sichuan, Ya'an, Baoxing, Fengtongzhai
Subathous sp.	JN561613	China: Yunnan, Lijiang, Mt. Wenbi
Athousius wudanganus (Kishii et Jiang, 1996)	JF713736	China: Hubei, Shennongjia
Denticollis davidi (Fairmaire, 1889)	JF713739	China: Sichuan, Ya'an, Baoxing, Qiaoqi
Hemicrepidius guizhouensis (Kishii et Jiang, 1996)	JF713751	China: Guangxi, Jinxiu, Tongmuzhen
Hemicrepidius variabilis (Fleutiaux, 1918)	JF713752	China: Sichuan, Leshan, Mabian
Hemicrepidius chinensis (Kishii et Jiang, 1996)	JF713750	China: Zhejiang, Zhenbu
Selatosomus albipubens (Reitter, 1910)	JF713790	China: Yunnan, Dali, Mt.Cangshan
Senodonia sculpticollis (Fairmaire, 1888)	JF713781	China: Guangdong, Shenzhen, Wutongshan
Senodonia quadricollis (Castelnau, 1836)*	EU677182	Jiang *et al*., 2009
Anostirus daimio (Lewis, 1894)	AB231223	Sagegami-Oba *et al*., 2007
Corymbitodes gratus (Lewis, 1894)	AB231231	Sagegami-Oba *et al*., 2007
Actenicerомorphus kurofunei (Miwa, 1934)	AB231213	Sagegami-Oba *et al*., 2007
Actenicerus orientalis (Candèze, 1889)	AB231214	Sagegami-Oba *et al*., 2007
Actenicerus pruinosus Motschulsky, 1861	AB231215	Sagegami-Oba *et al*., 2007
Selatosomus puerilis (Candèze, 1873)	AB231284	Sagegami-Oba *et al*., 2007
Pristilophus vagepictus (Lewis, 1894)	AB231271	Sagegami-Oba *et al*., 2007
Neopristilophus serrifer serrifer (Candèze, 1873)	AB231259	Sagegami-Oba *et al*., 2007
Denticollis nipponensis nipponensis Ôhira, 1973	AB231235	Sagegami-Oba *et al*., 2007
Athous subfuscus (O. F. Müller, 1767)	AB231226	Sagegami-Oba *et al*., 2007
Hemicrepidius secessus secessus (Candèze, 1873)	AB231247	Sagegami-Oba *et al*., 2007
Mucromorphus miwai miwai Kishii, 1962	AB231257	Sagegami-Oba *et al*., 2007
Limonius eximius Lewis, 1894	AB231252	Sagegami-Oba *et al*., 2007
Limoniscus niponensis (Lewis, 1894)	AB231250	Sagegami-Oba *et al*., 2007
Limoniscus vittatus (Candèze, 1873)	AB231251	Sagegami-Oba *et al*., 2007
Nothodes marginicollis (Lewis, 1894)	AB231260	Sagegami-Oba *et al*., 2007
Parapenia taiwana (Miwa, 1930)	AB231265	Sagegami-Oba *et al*., 2007
Pseudocsikia sp.	AB231275	Sagegami-Oba *et al*., 2007
Elaterinae		
Orthostethus sp.	JN561606	China: Sichuan, Leshan, Mabian
Ampedini nigrinus (Herbst, 1784)	JF713735	China: Sichuan, E'bian, Heizhugou
Megapenthes insignitus (Lewis, 1894)	JF713760	China: Hubei, Wuhan, Mt. Shizi
Gamepenthes versipellis (Lewis, 1894)	JF713747	China: Hubei, Guangshui, Daguisi

Continued Table 1

Taxa	GenBank No.	Collection location or reference
Procraerus sp.	JN561609	China: Guangdong, Mt. Nanling
Procraerus ligatus (Candèze, 1878)	JF713780	China: Guangxi, Mt. Shiwan
Glyphonyx rubricollis (Miwa, 1928)	JF713749	China: Guangxi, Guilin, Huajiang
Glyphonyx longipennis (Ôhira, 1966)	JF713748	China: Sichuan, Ya'an, Baoxing, Qiaoqi
Xanthopenthes granulipennis (Miwa, 1929)	JF713788	China: Guizhou, Mt. Fanjing, Panxi
Xanthopenthes robustus (Miwa, 1929)	JF713789	China: Hubei, Wuhan, Mt. Shizi
Silesis modestus (Candèze, 1878)	JF713784	China: Yunnan, Dali, Mt. Cangshan
Silesis rufipes (Candèze, 1896)	JF713786	China: Guangxi, J inxiu, Mt. Lao
Silesis absimilis (Candèze, 1836)	JF713782	China: Sichuan, Ya'an, Baoxing, Fengtongzhai
Silesis duporti (Fleutiaux, 1918)	JF713783	China: Hubei, Luotian, Tiantangzhai
Silesis okinawensis (Miwa, 1928)	JF713785	China: Hubei, Wuhan, Mt. Shizi
Parasilesis sauteri (Miwa, 1930)	JF713775	China: Sichuan, Ya'an, Baoxing, Fengtongzhai
Agriotes sericatus (Schwarz, 1891)	EU677181	Jiang *et al*., 2009
Agriotes angustatus (Miwa,1928)	JF713725	China: Guangxi, Mt. Mao'er
Agriotes subvittatus (Motschulsky, 1859)	JF713727	China: Yunnan, Dali, Mt. Weibao
Agriotes toukinesis (Fleutiaux, 1894)	JF713726	China: Yunnan, Nujiang, Fenshuiling
Chiagosnius dorsalis (Candèze, 1878)	JF713741	China: Guangxi, Lingui, Huangsha
Chiagosnius vittiger (Heyden, 1887)*	EU677179	Jiang *et al*., 2009
Chiagosnius obscuripes (Gyllenhal, 1817)*	EU677180	Jiang *et al*., 2009
Chiagosnius suturalis maculicollis (Candèze, 1863)*	EU677183	Jiang *et al*., 2009
Elater sieboldi (Candèze, 1873)	JF713746	China: Guangxi, Mt. Mao'er
Abelater pulcherus (Miwa, 1933	AB231212	Sagegami-Oba *et al*., 2007
Procraerus helvolus (Candèze, 1873)	AB231272	Sagegami-Oba *et al*., 2007
Procraerus kadesanus Ôhira, 1956	AB231273	Sagegami-Oba *et al*., 2007
Haterumelater bicarinatus bicarinatus (Candèze, 1873)	AB231246	Sagegami-Oba *et al*., 2007
Ampedus hypogastricus hypogastricus (Candèze, 1873)	AB231221	Sagegami-Oba *et al*., 2007
Ampedus optabilis optabilis (Lewis, 1894)	AB231222	Sagegami-Oba *et al*., 2007
Dalopius exilis Kishii, 1956	AB231234	Sagegami-Oba *et al*., 2007
Agriotes ogurae ogurae Lewis, 1894	AB231217	Sagegami-Oba *et al*., 2007
Agriotes pilosellus (Schönherr, 1817)	AB231238	Sagegami-Oba *et al*., 2007
Ectinus hidaensis Ôhira, 1998	AB231239	Sagegami-Oba *et al*., 2007
Ectinus insidiosus (Lewis, 1894)	AB231240	Sagegami-Oba *et al*., 2007
Ectinus sericeus sericeus (Candèze, 1878)	AB231241	Sagegami-Oba *et al*., 2007
Sadoganus babai Ôhira, 1956	AB231283	Sagegami-Oba *et al*., 2007
Parasilesis musculus musculus (Candèze, 1873)	AB231266	Sagegami-Oba *et al*., 2007
Glyphonyx bicolor bicolor Candèze, 1893	AB231244	Sagegami-Oba *et al*., 2007
Glyphonyx illepidius Candèze, 1873	AB231245	Sagegami-Oba *et al*., 2007
Sericus bifoveolatus (Lewis, 1894)	AB231285	Sagegami-Oba *et al*., 2007
Vuilletus crebrepunctatus (Nakane, 1959)	AB231287	Sagegami-Oba *et al*., 2007
Mulsanteus junior junior (Candèze, 1878)	AB231258	Sagegami-Oba *et al*., 2007
Orthostethus sieboldi sieboldi (Candèze, 1873)	AB231262	Sagegami-Oba *et al*., 2007
Parallelostethus sakishimensis (Ôhira, 1967)	AB231264	Sagegami-Oba *et al*., 2007
Dolerosomus gracilis (Candèze, 1873)	AB231237	Sagegami-Oba *et al*., 2007
Oestodinae		
Hemiops nigripes (Castelnau,1832)	JF713753	China: Hubei, Guangshui, Daguisi
Melanotinae		

Continued Table 1

Taxa	GenBank No.	Collection location or reference
Melanotus arctus (Candèze,1881)	JF713761	China: Hubei, Guangshui, Daguisi
Melanotus cribricollis (Faldermann, 1835)	JF713762	China: Guangdong, Mt. Nanling
Melanotus excelsus (Platia et Schimmel, 1991)	JF713763	China: Guangxi, Jinxiu, Mt. Huawang
Melanotus frequens (Miwa, 1930)	JF713764	China: Guangxi, Mt. Mao'er
Melanotus hourai (Kishii, 1898)	JF713765	China: Guizhou, Mt. Fanjing, Panxi
Melanotus lameyi (Fleutiaux, 1918)	JF713766	China: Guangxi, Jinxiu, Tongmuzhen
Melanotus lehmanni (Platia et Schimmel, 1991)	JF713767	China: Guangdong, Mt. Nanling
Melanotus melli (Platia et Schimmel, 1991)	JF713768	China: Guangdong, Zhaoqing, Heishiding
Melanotus nuceus (Candèze, 1881)	JF713769	China: Sichuan, Ya'an, Baoxing, Qiaoqi
Melanotus propexus (Candèze, 1860)	JF713770	China: Guangdong, Mt. Nanling
Melanotus splendidus (Platia et Schimmel, 1991)	JF713771	China: Hubei, Shennongjia
Melanotus tamsuyensis (Bates, 1866)*	EU677184	Jiang *et al*., 2009
Melanotus venalis (Candèze, 1860)	JF713772	China: Hubei, Shennongjia
Melanotus sp.	JN561605	China: Guangdong, Shenzhen, Mt. Wutong
Priopus angulatus (Candèze, 1860)	JF713779	China: Guangxi, Jinxiu, Mt. Huawang
Priopus pulchellus (Fleutiaux, 1923)	JN561607	China: Sichuan, Neijiang
Melanotus cete cete (Candèze, 1860)	AB231253	Sagegami-Oba *et al*., 2007
Melanotus correctus correctus (Candèze, 1865)	AB231254	Sagegami-Oba *et al*., 2007
Melanotus legatus legatus (Candèze, 1860)	AB231256	Sagegami-Oba *et al*., 2007
Melanotus koikei Kishii et Ôhira, 1956	AB231255	Sagegami-Oba *et al*., 2007
Priopus ferrugineipennis ferrugineipennis (Miwa, 1956)*	AB231270	Sagegami-Oba *et al*., 2007
Negastriinae		
Quasimus sp.	JN561612	China: Yunnan, Dali, Mt. Cangshan
Quasimus japonicus (Kishii,1979)*	AB231279	Sagegami-Oba *et al*., 2007
Quasimus ovalis (Candèze, 1873)	AB231280	Sagegami-Oba *et al*., 2007
Fleutiauxellus cruciatus (Candèze, 1873)*	AB231242	Sagegami-Oba *et al*., 2007
Fleutiauxellus curatus curatus (Candèze, 1873)	AB231243	Sagegami-Oba *et al*., 2007
Oedostethus telluris (Lewis, 1879)*	AB231261	Sagegami-Oba *et al*., 2007
Yukoana carinicollis (Lewis, 1879)*	AB231288	Sagegami-Oba *et al*., 2007
Cardiophorinae		
Dicronychus extractus (Fleutiaux, 1892)	JF713745	China: Guizhou, Mt. Fanjing, Panxi
Platynychus nothus (Candèze, 1865)	JF713792	China: Guangxi, Jinxiu, Tongmuzhen
Paracardiophorus subaeneus (Fleutiaux, 1902)	JF713774	China: Sichuan, Ya'an, Baoxin, Longdong
Phorocardius unguicularis (Fleutiaux, 1865)	JF713778	China: Guangxi, Lingui, Huangsha
Phorocardius comptus (Candèze, 1860)	JF713777	China:Yunnan, Dali, Mt. Cangshan
Displatynychus adjutor (Candèze, 1873)*	AB231236	Sagegami-Oba *et al*., 2007
Ryukyucardiophorus loochooensis (Miwa, 1934)*	AB231282	Sagegami-Oba *et al*., 2007
Cardiophorus niponicus Lewis, 1894	AB231228	Sagegami-Oba *et al*., 2007
Cardiophorus pinguis Lewis, 1894	AB231229	Sagegami-Oba *et al*., 2007
Paracardiophorus nakanei hondoensis Ôhira, 1997	AB231263	Sagegami-Oba *et al*., 2007
Platynychus nothus (Candèze, 1865)	AB231269	Sagegami-Oba *et al*., 2007
Outgroup		
Artematopidae		
Eurypogon japonicus Sakai, 1982*	AB232643	Sagegami-Oba *et al*., 2007
Buprestidae		
Aphanisticus yasumatsui (Kurosawa, 1954)*	AB232646	Sagegami-Oba *et al*., 2007

Continued Table 1

Taxa	GenBank No.	Collection location or reference
Chrysodema manillarum (Thomson, 1879)*	AB232644	Sagegami-Oba *et al*., 2007
Coraebus quadriundulatus (Motschulsky, 1866)*	AB232645	Sagegami-Oba *et al*., 2007
Cantharidae		
Lycocerus suturellus suturellus (Motschulsky, 1860)	AB232647	Sagegami-Oba *et al*., 2007
Dascillidae		
Dascillus cervinus (Linnaeus, 1758)*	AJ810783	Korte *et al*., 2004
Eucnemidae		
*Bioxylus sp.**	AB232648	Sagegami-Oba *et al*., 2007
Lampyridae		
Drilaster axillaris (Kiesenwetter, 1879)*	AB232649	Sagegami-Oba *et al*., 2007
Lucidina biplagiata (Motschulsky, 1866)*	AB232650	Sagegami-Oba *et al*., 2007
Luciola cruciata (Motschulsky, 1854)*	AB232651	Sagegami-Oba *et al*., 2007
Lycidae		
Dilophotes atrorufus (Kiesenwetter, 1879)*	AB232652	Sagegami-Oba *et al*., 2007
Lyponia quadricollis (Kiesenwetter, 1879)*	AB232653	Sagegami-Oba *et al*., 2007
Omethidae		
Drilonius striatulus (Kiesenwetter, 1874)*	AB232654	Sagegami-Oba *et al*., 2007
Throscidae		
Trixagus turgidus Hisamatsu, 1985	AB232655	Sagegami-Oba *et al*., 2007

PCR amplification and DNA sequencing

The 28S rDNA (800–930 bp) region was amplified via PCR using a Mastercycler Gradient 5331 thermal cycler (Eppendorf, Hamburg, Germany). The reaction was conducted in a 25-μL volume using the primers 28S-01 (5′-GAC TAC CCC CTG AAT TTA AGC AT-3′) and 28SR-01 (5′-GAC TCC TTG GTC CGT GTT TCA AG-3′) (Hunt *et al*. 2007; Hassanin *et al*. 1998). The reaction conditions were: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 51°C for 15 s, extension at 72°C for 2.5 min, and a final extension at 72°C for 10 min. The amplification products were purified using an AxyPrep DNA gel extraction kit (Axygen, Shanghai, China).

Signal detection development

To compute the substitution rates, a plot was constructed using the distances between pairs of sequences and the numbers of transitions (TS) and transversions (TV). Three models, the Kimura two-parameter distance (Philips & Simon 1995), the Tamura three-parameter distance (Kimura 1980), and the maximum composite likelihood distance (Tamura 1992, Tamura & Nei 1993) were used to draw the plots. The degree of saturation of a sequence was evaluated based upon the trend line of the plot.

Sequence alignment

The sequences were initially aligned using three different software programs under different settings: MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/software/) (Katoh *et al*. 2002, 2005; Katoh & Toh 2010), Clustal X v. 2.0 (http://www.clustal.org/) (Thompson *et al*. 1997), and MUSCLE v. 3.8.31 (http://www.ebi.ac.uk/Tools/msa/muscle/) (Edgar 2004). MAFFT 6.864b using the G-INS-i setting was ultimately chosen for multiple alignment

because of the stable phylogenetic tree topology in the subsequent analyses. Gblocks v. 0.19b (http://molevol.cmima.csic.es/castresana/Gblocks.html) (Castresana 2000) was used to examine the resulting alignments, although the removal of blocks was not considered necessary to construct the phylogenetic trees. The parameters were as follows: minimum number of sequences for a conserved position, a flank position, and a contiguous non-conserved position were 84, 141, and 8, respectively; minimum length of a block was 10; gap position was not allowed. Ultimately, 45% of the alignment was removed.

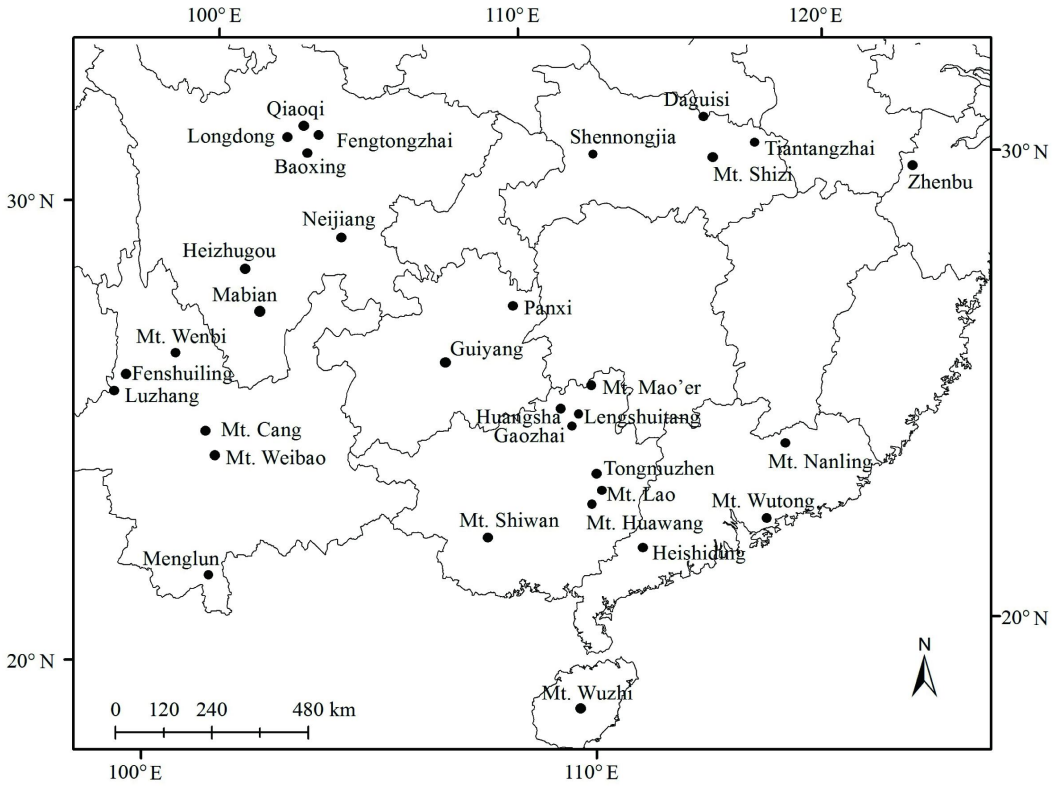

Figure 1. Regions where Elateridae samples were collected.

Phylogenetic analysis

We reconstructed evolutionary trees for Elateridae based on the 28S rDNA sequences of 80 species we collected and of 100 other species (both ingroup and outgroup). We used an 812-nucleotide fragment that was consistent across all 166 elaterid species; 433 positions were variable, and 368 were parsimony-informative. The 14 outgroup taxa represented nine different families.

Phylogenetic trees were inferred using the NJ, ML, MP, and BI methods. The NJ, ML, and MP analyses were carried out using MEGA X (http://www.megasoftware.net/) (Kumar *et al*. 2018), which was also used to compute the nucleotide composition. The BI tree was reconstructed with MrBayes v. 3.2 (http://www.mrbayes.net/) (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). All positions containing gaps and missing data were eliminated from the analysis.

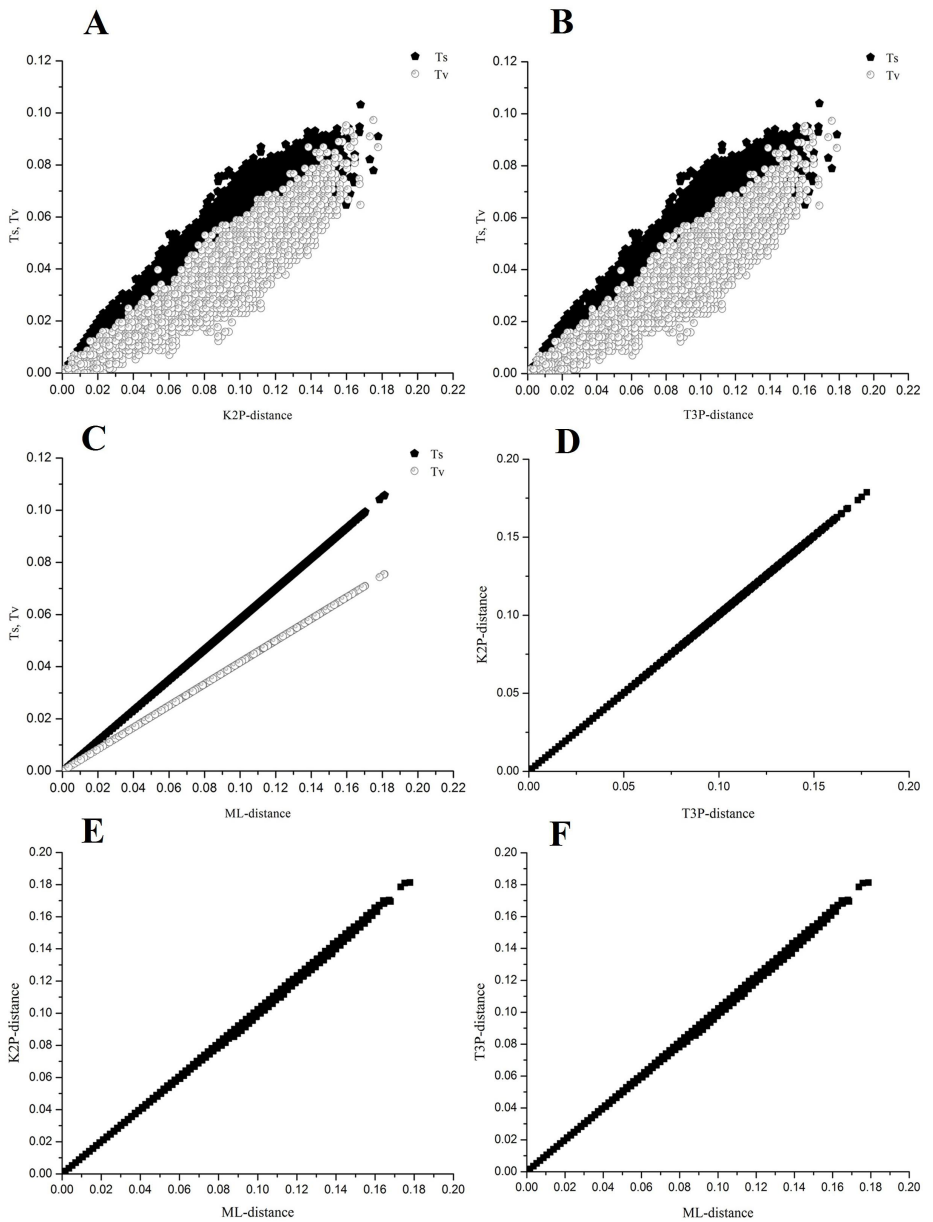

Figure 2. Signal detection plots based upon the 28S rDNA sequences of different Elateridae beetle specimens. A. Trend line of the Kimura two-parameter distance according to the numbers of transitions (TS) and transversions (TV); B. Trend line of the Tamura three-parameter distance according to the numbers of TS and TV; C. Trend line of the maximum composite likelihood distance according to the numbers of TS and TV; D. Regression line of the number of substitutions based upon Kimura's two-parameter distance against the Tamura three-parameter distance; E. Regression line of the number of substitutions based upon the Kimura two-parameter distance against the maximum composite likelihood distance; F. Regression line of the number of substitutions based upon the Tamura three-parameter distance against the maximum composite likelihood distance.

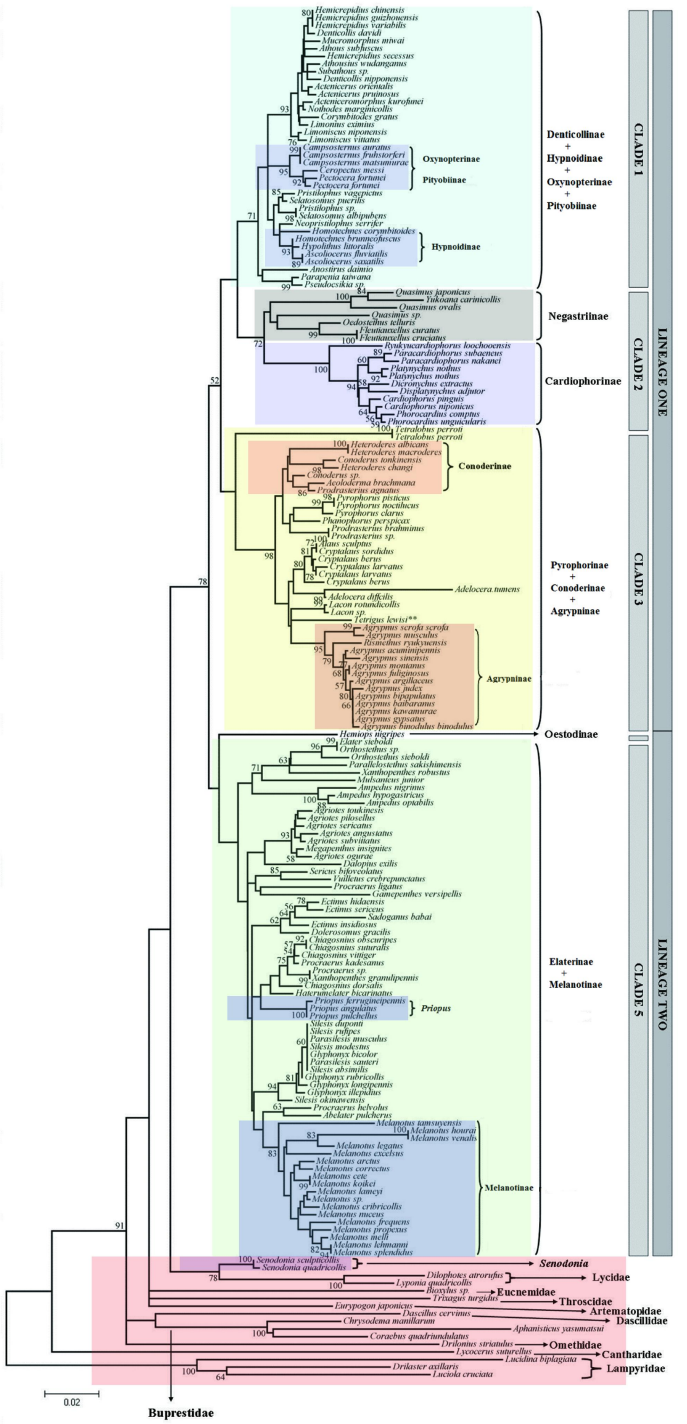

Figure 3. Neighbor-joining phylogenetic tree of Elateridae and 14 outgroup taxa based on the analysis of the 28S rDNA data set. Neighbor joining consensus tree of 1000 bootstrap replicates. Bootstrap support values > 50% are shown next to the branches; branches with < 50% bootstrap support are collapsed. Branch lengths represent evolutionary distances computed using the Tamura-Nei method (base substitutions per site).

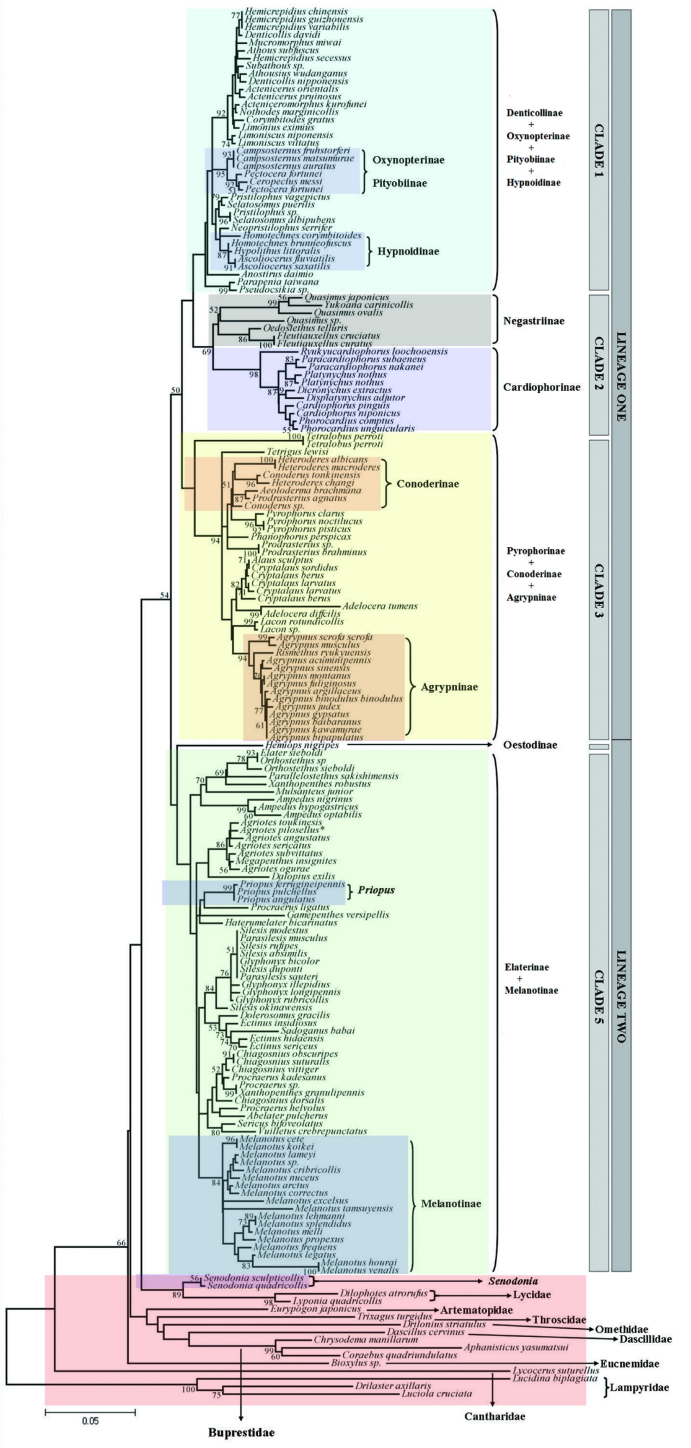

Figure 4. Maximum-likelihood tree based on the GTR + I + G model of substitution (−lnL = 8841.1514). Bootstrap support (1000 replicates) is shown next to the branches. Branch lengths represent the number of substitutions per site.

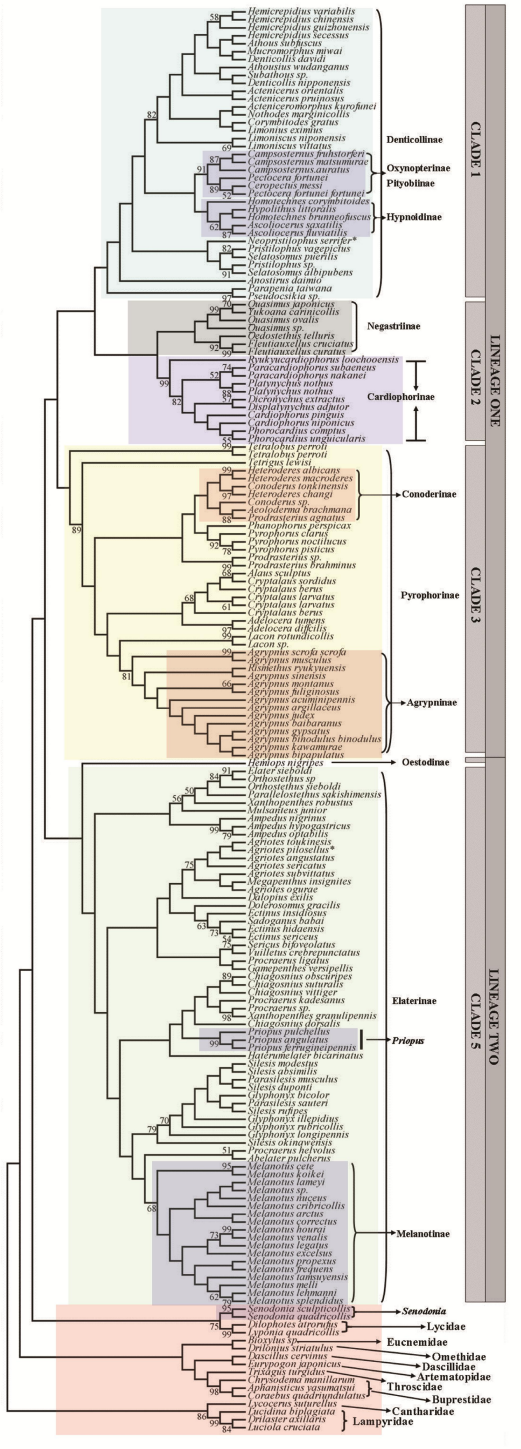

Figure 5. One of 87 maximum parsimony trees (length = 1710). For parsimony-informative sites, the consistency index was 0.281459, the retention index was 0.755735, and the composite index was 0.212708 (0.233350 for all sites). Bootstrap support (1000 replicates) is shown next to the branches.

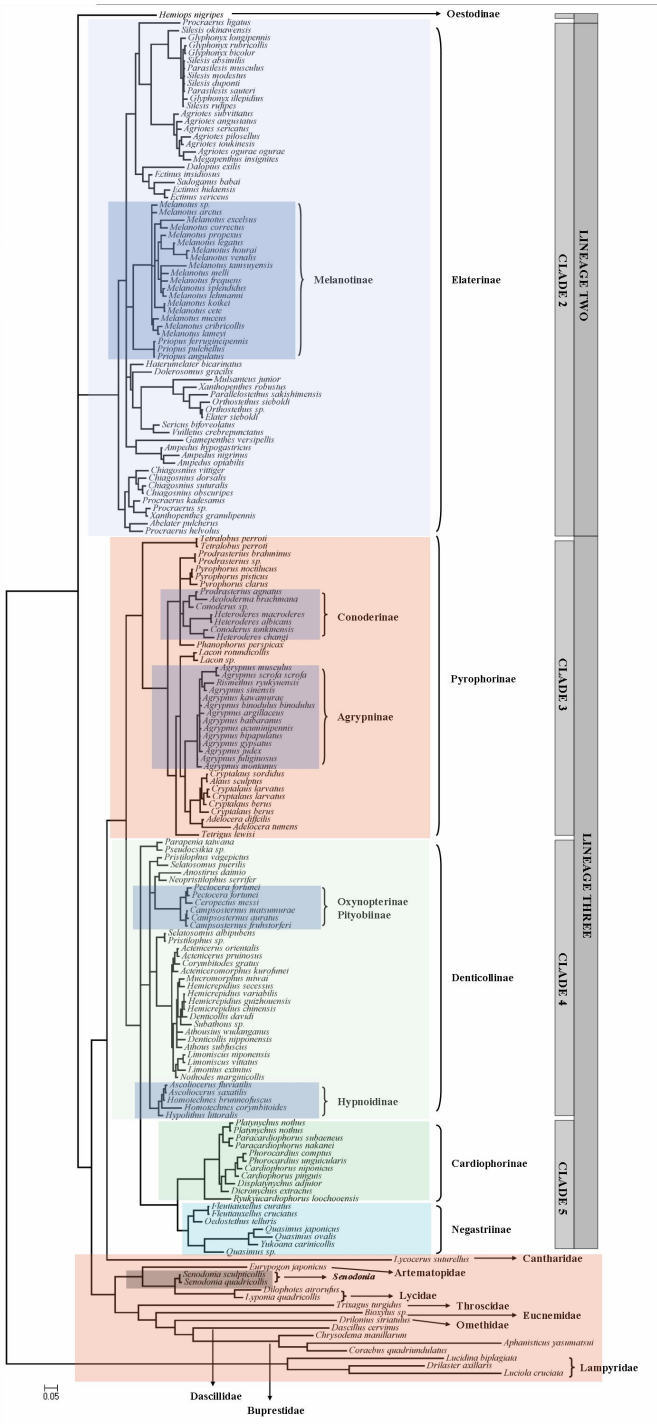

Figure 6. Bayesian inference tree using the following parameters: nucleotide state frequencies fixed as equal; invgamma; two independent runs of four MCMC chains for 13,640,000 generations; trees were sampled every 5,000 generations for a total of 2,728 trees, with 2,046 trees discarded as burn-in; average standard deviation of split frequencies was 0.00998.

We used the Kimura two-parameter model with uniform rates to conduct the NJ analyses based on the number of nucleotide substitutions per site (Saitou & Nei 1987). ML analyses were performed using the GTR + I + G model (Rodriguez *et al*. 1990). The phylogenetic trees were examined using 1,000 bootstrap replicates with 10 random additions per replicate and the Nearest-Neighbor-Interchange algorithm (Felsenstein 1985). This model of sequence evolution was selected using MODELTEST v. 3.7 (http://www.molecularevolution.org/software/phylogenetics/modeltest) (Posada & Crandall 1998). For MP analyses, we used the Close-Neighbor-Interchange method with 10 random additions per replicate. Branch support for all phylogenetic trees was determined using 1,000 bootstrap replicates. For BI, MrBayes v.3.2 was employed through parsimony analysis using Bayesian approaches. Two simultaneous runs of four Markov chain Monte Carlo chains each were run for 13.64 million generations using random trees as starting points and sampling every 5,000 generations. We got a total of 27, 28 trees and discarded 2,046 trees as "burn-in".

Results

rDNA sequences

The newly-acquired 28S rDNA sequences of 80 species from 12 subfamilies of Chinese Elateridae were deposited in GenBank under the accession numbers JF713723–JF713792 and JN561604–JN561613. The base frequencies were as follows: A, 17.1–27.1% (mean ± SE = 18.7 ± 0.08%); T, 15.4–20.4% (17. 6 ± 0.09%); G, 26.8–31.8% (34.6 ± 0.05%); and C, 23.3–23.5% (29.1 ± 0.1%). No significant differences were identified in nucleotide composition among species. The G + C contents were very similar (63.7% ± 0. 1%) among the ingroup taxa. These results agree with those in a previous report (Sagegami-Oba *et al*. 2007).

Signal detection development

Figure 2 shows that the base substitutions were not saturated. Therefore, these sequences could be used for phylogenetic analysis.

Phylogenetic analysis

The neighbor-joining (NJ), maximum-parsimony (MP), and maximum-likelihood (ML) analyses consistently produced five distinct basal clades of Elateridae in two lineages (Fig. 3 – Fig. 5). The first lineage comprised two branches that further subdivided into three clades (Clades 1–3) and the second lineage comprised two clades (Clades 4–5). The ingroup structure resulting from Bayesian inference (BI) was divided into five major clades in three lineages. The first two lineages only had one clade each (Clades 1 and 2, respectively) and the third lineage comprised three clades (Clades 3–5) (Fig. 6).

In the NJ, MP, and ML trees, Clade 1 was comprised of Pityobiinae, Oxynopterinae, Denticollinae and Hypnoidinae subfamilies. Pityobiinae and Oxynopterinae were reciprocally non-monophyletic and were made paraphyletic by having Denticollinae and Hypnoidinae nested within. The main difference between the MP tree (Fig. 5) and the NJ (Fig. 3) and ML (Fig. 4) trees was that, in the former, *Neopristilophus serrifer* fell within Denticollinae rather than Hypnoidinae, as in the two latter trees. In addition, Hypnoidinae formed a clade in the MP tree but not in the NJ and ML trees. In all three trees, Pityobiinae and Oxynopterinae, each

represented by a single branch, were sister to one another; this clade was grouped with Hypnoidinae in the MP tree but not in the NJ and ML trees. Only two subfamilies, Negastriinae and Cardiophorinae, were included in Clade 2, and each formed a clade in all three trees. Clade 3 consisted of three subfamilies, Agrypninae, Pyrophorinae, and Conoderinae, and differed somewhat among the three methods. For instance, *Tetrigus lewisi* was monophyletic in the ML and MP trees but not the NJ tree. Similarly, *Rismethus ryukyuensis*, in Agrypninae, was sister to *Agrypnus sinensis* in the MP analysis but was distinct from that species in the NJ and ML trees. This study supports the independence of the Oestodinae (Clade 4) despite its close relationship with Elaterinae (Clade 5). Elaterinae and Melanotinae together form Clade 5, with some differences among methods. For instance, the genera *Priopus* and *Chiagosnius* were grouped together in the NJ and MP trees but not the ML tree.

In the BI tree, the phylogenetic positions of all the subfamilies differed from those in the NJ, ML, and MP trees. Oestodinae formed an individual lineage as the first clade above the phylogenetic tree. Elaterinae and Melanotinae formed Clade 2. Clade 3 included Agrypninae, Pyrophorinae, and Conoderinae. The fourth clade included Pityobiinae, Oxynopterinae, Denticollinae, and Hypnoidinae. The fifth clade was formed by Negastriinae and Cardiophorinae. In the BI tree, all of the subfamilies were monophyletic. Although their phylogenetic positions were different, all five clades in the BI tree contained the same taxa as the five clades in the NJ, MP, and ML analyses.

Discussion

Phylogeny of Elateridae

In this study, we constructed a phylogenetic tree for 166 species of Elateridae using 28S rDNA gene sequences. Fourteen outgroup species were used and Lampyridae was chosen to root the tree because it was phylogenetically most distant to Elateridae. Our phylogenetic results mostly supported those previously reported by Sagegami-Oba *et al*. on Japanese Elateridae (2007). In both studies, the elaterid species were divided into two lineages, the first of which had an identical structure in the study of Sagegami-Oba *et al*. to our NJ, ML, and MP trees. However, Sagegami-Oba *et al*. reported that only one clade (Elaterinae + Melanotinae) was grouped into their lineage 2, but not Oestodinae, as in our findings. In the BI tree, Oestodinae even formed an independent lineage. Thus, the results of the current study both confirm and supplement those of the previous study. However, we only analyzed one species of Oestodinae, and further analyses with additional samples will be required to confirm the classification of this group.

In the NJ, ML, and MP analyses, there were four subfamilies (Denticollinae + Hypnoidinae + Oxynopterinae + Pityobiinae) in Clade 1 (Figs. 3 – Fig. 5). Clade 4 in the BI tree contained the same four subfamilies but in a different position (Fig. 6). Pityobiinae and Oxynopterinae were grouped together with strong statistical support, consistent with the study of Sagegami-Oba *et al*. (2007). Based on adult external morphology, Stibick also suggested a close relationship between these two subfamilies, given that the meso- and metasterna remain unsutured in the adults of both (Stibick 1979). Moreover, Kundrata and Bocak proposed to integrate Pityobiinae, Hypnoidinae, and Oxynopterinae as tribes Pityobiini, Hypnoidini and

Oxynopterini within the Denticollinae subfamily (2011), suggesting their close evolutionary relationships – this branching order was not supported by our results. In contrast, the phylogenetic analysis conducted by Kishii based on adult external morphology grouped Denticollinae, Hypnoidinae, and Pityobiinae together in a clade that did not include Oxynopterinae because of the connation of the meso- and metasterna in adults of the latter (1987).

Cardiophorinae and Negastriinae together formed Clade 2 in the NJ, ML, and MP trees, but their positions within Elateridae were different from those based on traditional morphological classifications. Kishii (1987) and Stibick (1979) placed these two subfamilies basally within Elateridae given their unique morphological features rather than at a derived position as shown in our NJ, ML and MP trees. However, in our BI tree, these subfamilies (Clade 5; Fig. 6) were placed at the base of the family, in accord with the results of Kishii and Stibick. These authors also proposed a close relationship between Cardiophorinae and Negastriinae, which fits well with our results. Remarkably, the relationships within this clade in our study agreed with those of a previous molecular phylogeny (Sagegami-Oba *et al*. 2007).

The subfamilies Agrypninae, Conoderinae, and Pyrophorinae formed the third clade in all four of our analyses. Agrypninae and Conoderinae, both of which were monophyletic, clustered within Pyrophorinae. Our results differ from some previous reports. For instance, our findings support the existence of all three subfamilies, but some entomologists have suggested that these species be lumped into two subfamilies (Agrypninae and Conoderinae) based on whether the mesocoxal cavity opens into the mesepisternum (Kishii 1987). However, this system poses some contradictions. For example, the basic distinction between Conoderinae and Pyrophorinae is the presence of membranous lobes on the ventral side of the fourth tarsal segment in the former but not the latter. However, Tetralobini within Pyrophorinae were named precisely because of these membranous lobes on their tarsi. Other researchers have categorized the three subfamilies into one (Pyrophorinae) based upon the presence of setae at the base of the claws in adults and the lack of mandibles with teeth in the larvae (Stibick 1979). The classification of these species in a single subfamily (Pyrophorinae or Agrypninae) was previously supported by morphological features (Ohira 1962; Jeannel & Paulian 1944; Kishii 1987) and molecular systematics (Bocakova *et al*. 2007; Kundrata & Bocak 2011). The genus *Tetralobus*, which was not included in the previous study, has been categorized morphologically within Pyrophorinae, whereas in our analyses *Tetralobus perroti* was nested within Clade 3 (Fig. 3 – Fig. 6). In contrast, paraphyly is indicated between *Tetralobus* and other taxa of Pyrophorinae. Notably, the body of *T. perroti* is larger than that of other species and the 1st through 4th tarsi are covered with broad lobes, unlike the simple 4th tarsus in other species of Pyrophorinae. So the position of *T. perroti* determined by our analyses is not unreasonable. Based upon our molecular analysis, Oestodinae is closely related to Elaterinae, consistent with traditional morphological classification of Stibick (1979). According to his morphological analyses, Oestodinae was nested within Elaterinae, but in our study, it forms an independent clade. This placement is partly supported by morphology in that the frontal ridge on the forehead of Oestodinae is interrupted, while that of Elaterinae or Melanotinae is complete, although all three have hypognathous mouthparts. Remarkably, the placement of Oestodinae has not received much attention in previous molecular phylogenetic studies. However, we only analyzed one species of Oestodinae; more samples will be required to

confirm our conclusion.

Elaterinae and Melanotinae were grouped together in Clade 5 in our NJ, ML, and MP trees and in Clade 2 in the BI tree. The species relationships within Elaterinae are largely consistent those previously reported based on the morphology of both adults and larvae (Ohira 1962; Stibick 1979; Kishii 1987). Nonetheless, small differences exist. Stibick once defined the new subfamily Aplastinae which, together with its sister group Oestodinae, was placed within the Elaterinae (Stibick 1979). In any case, our results and those of others support the hypothesis that Elaterinae is closely related to Melanotinae (Kishii 1987). The taxa in our Clade 5 agree with the results of a previous molecular analysis (Sagegami-Oba *et al*. 2007). However, we found that *Priopus* was a member of Elaterinae and *Melanotus* was monophyletic within Melanotinae, while *Priopus* and *Melanotus* were both members of Melanotinae in the ML tree of Sagegami-Oba *et al*. (2007). In addition, they found an independent tribe (Agriotini) that we did not recover. Further studies will be required to resolve these divergent findings.

Monophyly of Elateridae

We analyzed the 28S rDNA sequences of 166 species of Elateridae belonging to different subfamilies. Some specimens of the same species were sampled from different geographic locations, so their phylogenetic relationships were not influenced by geographic factors. The 28S rDNA gene is thought to provide clear phylogenetic evidence because of its conserved nature (Bocakova *et al*. 2007; Kundrata & Bocak 2011; Sagegami-Oba *et al*. 2007).

Our phylogenetic analyses of 28S rDNA sequences using NJ, ML, MP, and BI methods all strongly support the monophyly of Elateridae, with the exception of *Senodonia*, which was not placed with Elateridae in our results. The relationship between *Senodonia* and Lycidae should be more closely examined. Notably, this genus is mainly distributed on the Indo-China Peninsula, yet no molecular phylogenetic studies of Elateridae have included it. *Senodonia* was traditionally included in Denticollinae, but it forms a clade with Lycidae in our study. Morphologically, *Senodonia quadricollis* or *Senodonia sculpticollis* resemble Elateridae in the distinctive prosternal process, the mesosternal cavity, and the well-known defense "clicking" mechanism. The mesocoxal cavities of the two *Senodonia* species open to both the mesepimeron and mesepisternum, similar to Denticollinae. In addition, their elytra and exoskeletons are relatively hard, unlike the soft elytra and bodies of Lycidae. Also, the elytra of Lycidae are larger than the body, while those of *Senodonia* only cover the hind part of the body, as in Elateridae. Therefore, *Senodonia* shares many morphological similarities with Elateridae. But in our analyses it was more phylogenetically related to Lycidae than to other members of Elateridae. We ruled out the possibility of long branch attraction by rooting the trees with different outgroups (data not shown).

We analyzed 28S rDNA sequence data from 12 previously-established subfamilies of Elateridae. Our phylogenetic results should help to clarify the classification of this family. They suggested that Hypnoidinae, Oxynopterinae, and Pityobiinae be classified as tribes of the subfamily Denticollinae; Agrypninae and Conoderinae be treated as tribes of Pyrophorinae; and Melanotinae be merged into Elaterinae. These taxonomic changes would yield six subfamilies —Denticollinae, Pyrophorinae, Elaterinae, Oestodinae, Cardiophorinae, and Negastriinae — in agreement with the results of Kundrata and Bocak (2011). The outgroup

taxa Lycidae, Cantharidae and Lampyridae have been considered subfamilies within Cantharoidea. Kasap and Crowson (1975) [Crowson 1961 is the single author article, Kasap and Crowson is 1975] and Lawrence *et al*. (1995) once suggested a close relationship between the Elateroidea and Cantharoidea. In our results, although the species of Cantharoidea were distinct, they grouped with Elateroidea.

Conclusions

There were some limitations in our study. We only used one molecular maker to reconstruct the phylogenetic trees, and we did not sample many species of Oestodinae or *Senodonia*. Also, the geographical limitations of our click beetle samples limit the systematic conclusions we can draw. Elateridae is widely considered to be monophyletic, a conclusion that is strongly supported by molecular systematic studies. More robust phylogenetic conclusions will require the sampling of more species and the use of additional molecular markers. The present work suggests that stronger support for the phylogeny of Elateridae can be obtained by applying molecular analyses to a broader range of species groups. We hope that phylogenetic trees can provide real insights into the evolution of Elateridae (Anisimova *et al*. 2013). Clearly, using only two species of *Senodonia* is insufficient, and the phylogenetic position of this genus requires additional research.

Acknowledgements

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